GUIDELINES AND RESOURCES

Interim Guidance for Collection of Diagnostic Specimens from Persons with Suspect Monkeypox

The Centers for Disease Control and Prevention (CDC) is working with local and state health departments and public health laboratories to test specimens from humans and animals with suspected monkeypox infection. This document provides interim recommendations for the safe collection of human clinical specimens for monkeypox testing.

Reporting of Suspect Human Monkeypox Cases

Report possible human cases of monkeypox to your local hospital epidemiologist and/or infection control personnel, who will contact your state health department. The state health department will contact the Centers for Disease Control and Prevention (CDC), if appropriate. Consultation with the state epidemiologist (www.cste.org/members/state_and_territorial_epi.asp) and state health laboratory (www.aphl.org/public_health_labs/index.cfm) is necessary before sending specimens to CDC. After CDC has been contacted (at www.cdc.gov/ncidod/monkeypox/pdf/specimenguide.pdf) for approval to send the specimens, the samples should be collected in the manner outlined below and placed in an appropriate biosafe shipping container.

Protection of Personnel

Specimen collection personnel. Personnel who collect specimens should use personal protective equipment (PPE) in accordance with recommendations for Standard, Contact, and Airborne Precautions (www.cdc.gov/ncidod/monkeypox/infectioncontrol.htm). Current recommendations for smallpox vaccination of health-care workers also should be considered (see: www.cdc.gov/ncidod/monkeypox/treatmentguidelines.htm).

Laboratory personnel. For guidance regarding the use of smallpox vaccine for laboratory personnel who potentially might handle specimens from patients with monkeypox, see Interim Biosafety Guidelines for Laboratory Personnel at: www.cdc.gov/ncidod/monkeypox/labbiosafetyguide.htm

Sharps injury prevention. When possible, use plastic rather than glass materials for specimen collection and processing (e.g., blood and capillary tubes, bottles, slides) and devices with sharps injury prevention features. Dispose all sharps in a sharps container immediately after use.

Processing of routine clinical specimens. For information about laboratory precautions when processing routine clinical specimens, see Interim Biosafety Guidelines for Laboratory Personnel at www.cdc.gov/ncidod/monkeypox/labbiosafetyguide.htm

Collection of Specimens for Monkeypox Diagnosis

This section provides guidance for the collection of various clinical specimens during the rash phases of monkeypox as well as the prodromal and convalescent stages. Samples should be collected in the manner

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specified below and placed in an appropriate biosafe shipping container, please see this URL: www.cdc.gov/ncidod/monkeypox/pdf/specimenguide.pdf

I. Specimen Collection Supply List

Some or all of the following materials will be required for specimen collection from each patient:

Blood collection

- 5- or 10-cc syringe with 18- or 20-gauge needle (pediatrics: smaller syringe and smaller gauge needle may be necessary)
- 1 vacutainer holder
- 2 vacutainer needles (20 x 1.5 in.)
- 1 10-cc marble-topped vacutainer tube, **or** 1 10-cc yellow-topped serum separator tube for serum collection (plastic tube preferable)
- 1 5-cc purple-topped tube (potassium EDTA anticoagulant) for whole blood buffy coat collection for viral isolation (plastic tube preferable)
- Styrofoam tube protectors

Lesion collection: macular, papular, vesicular, pustular,scabs

- Disposable scalpel (1) with No. 10 blade
- Sterile 26-gauge needles (several)
- Punch biopsy kit; 2.5- (pediatric), 3.5- or 4-mm
- 10% buffered formalin
- Needle driver
- Suture
- Suture removal kit
- Sterile dry polyester or Dacron swabs (e.g., Catch-All sample collection swabs, catalog no. QEC091H from Epicentre in Madison, Wisconsin (Epicentre www.epicentre.com/main.asp) (4-8)
- Clean plastic or glass microscope slides (4)
- Plastic single-slide holders (4)
- Formvar/carbon-coated mesh electron microscopy grids (2-4) available at www.emsdiasum.com/ems, catalog no. FCF400-Cu; sterilize under a UV light for 10 minutes prior to use
- Electron microscopy quality forceps; available at www.emsdiasum.com/ems, catalog no. 72750-F
- Electron microscopy grid box, available at www.emsdiasum.com/ems, catalog no.71150
- Sterile screw-capped plastic vials (1.5 to 2.0 ml) (Sarstedt with O-ring)
- Parafilm

Oropharyngeal specimen collection

- Polyester or Dacron swabs (e.g., Catch-All sample collection swabs, catalog no. QEC091H from Epicentre in Madison, Wisconsin (Epicentre www.epicentre.com/main.asp)

Other

- Sharps container
- Disposable biohazard bags

Disclaimer: Names of vendors or manufacturers are provided as examples of suitable product sources; inclusion does not imply endorsement by the Centers for Disease Control and Prevention, Department of Health and Human Services.

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II. Specimen Types and Labeling Procedure

Suitable specimens for virologic tests are:

- Vesicular or pustular tissue and fluid
- Scabs
- Biopsy tissues
- Throat swabs and whole blood

Suitable specimen for serologic tests are:

- Serum

Specimen Labeling: Label ***all*** tubes, vials, microscope slides, and EM grid holders with the following:

- Patient name
- Date and time of collection
- Source of specimen (vesicle, pustule, or scab)
- Date of birth of patient (for cross-referencing of specimens)
- Name or initials of person collecting specimen
- If patient is hospitalized, include hospital and identification numbers (e.g., medical record number, surgical pathology number)
- State ID number or CDC monkeypox ID number.

III. Pre-collection Procedures

- Perform hand hygiene and put on the recommended personal protective equipment (see Updated Interim Infection Control and Exposure Management Guidance in the Health-Care and Community Setting for Patients with Possible Monkeypox Virus Infection at the following URL: www.cdc.gov/ncidod/monkeypox/infectioncontrol.htm
- When possible, obtain digital photographs of lesions that will be sampled to send with specimens.

IV. Stepwise Collection Procedures for Patients with *Acute Symptoms* of Monkeypox

Rash lesions: Macular*, papular*, vesicular, or pustular lesions

1. Sanitize skin with an alcohol wide, allow to dry.
2. Use scalpel (or a sterile 26-gauge needle) to open, and remove, the top of the vesicle or pustule. Do not send the scalpel or sharp. Dispose of in appropriate biohazard container and dispense.
3. Place the skin of the vesicle top into a 1.5- to 2-mL sterile screw-capped plastic tube with O-ring. Leave the material dry.
4. Scrape the base of the vesicle or pustule with the blunt edge of the scalpel, or with the wooden end of an applicator stick or swab.
5. Smear the scrapings onto a clean glass microscope slide.
6. Apply a microscope slide to the vesicular fluid multiple times, with progressive movement of the slide, to make a touch prep.
7. If a slide is not available, swab the base of the lesion with a polyester or cotton swab place in a screw-capped plastic vial, break off swab handle and screw on lid. ***Do not*** *add transport medium to the vial*.
8. If available, lightly touch "shiny side" of an electron microscope grid to the unroofed base of the lesion. Repeat this procedure two more times, varying the pressure applied to the unroofed lesion (lighter or firmer pressure). Place in gridbox and record which slot is used for each patient specimen.
9. Allow slides and grids to air dry for approximately 10 minutes.

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10. Store slides in plastic slide holders for shipping. Parafilm may be used to wrap the slide holder to prevent accidental opening. Store slides from different patients in ***separate*** plastic slide holder to prevent cross-contamination.

*For patients with macular or papular rash, sample lesion via biopsy and acquire tonsillar tissue swab, serum, and whole blood as described below.

Scab lesion

1. Sanitize skin with an alcohol wide, allow to dry.
2. Use a 26-gauge needle to pick/pry off as many scabs as possible (at least four).
3. Place two scabs each in screw-capped plastic 1.5- to 2-mL vials.
4. Use appropriate sterile technique.

Lesion biopsy

1. Use appropriate sterile technique and skin sanitation.
2. Biopsy lesion (2) with 3.5- or 4-mm biopsy punch (at least 2.5 mm; 3.5-4 mm preferable).
3. Place one biopsy specimen in formalin.
4. Place one biopsy specimen in a 1.5- to 2-mL screw-capped vial. ***Do not*** *add any fluid.*

Tonsillar tissue swab

Swab or brush posterior tonsillar tissue and break off end of applicator into a 1.5- to 2-mL screw-capped tube. ***Do not*** *add transport medium.* ***Use polyester or Dacron swab.***

Blood samples

1. Obtain an acute-phase serum sample. Collect 7 to 10 cc of patient blood into a marble-topped tube, or yellow-topped serum separator tube. Spin samples to separate serum. Save the serum in at least two aliquots. Label tubes as acute serum, with other information listed in Specimen Labeling (e.g., case ID number, date of collection). Store half of the serum at the state health department and send half to CDC for testing.
2. Obtain whole blood sample. Collect 3 to 5 cc of blood into a purple-topped tube. Gently mix blood with anticoagulant in tube to prevent clotting. Label tube as whole blood, with other information listed in Specimen Labeling (e.g., case ID number, date of collection). Send the tube with whole blood to CDC for testing.
3. Obtain a convalescent-phase serum sample (4 to 6 weeks after collection of acute-phase serum). Collect 7 to 10 cc of patient blood into a marble-topped tube, or yellow-topped serum separator tube. Spin samples to separate serum. Save the serum, and label the tube as convalescent serum, with other information listed in Specimen Labeling (e.g., case ID number, date of collection). Send convalescent-phase serum, together with the remainder of the acute-phase sample stored at the health department, to CDC for paired sera testing.

Note: Blood samples from person with severe, dense rash may be difficult to draw as the skin may slough off. A central line may be needed for access in cases where a peripheral blood draw is difficult.
Note: For pediatric patients, a minimum of 1 cc of whole blood is needed for testing. If possible, collect at least 1 cc in each of an EDTA and clotting tube. However, if only 1 cc can be obtained, use a clotting tube for collection.

V. Stepwise Collection Procedure for Patients Identified 6-8 Weeks After Onset of Rash Illness Suspected to be Monkeypox

Blood samples

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1. Obtain an initial serum sample when patient is first identified. Collect 7 to 10 cc of patient blood into a marble-topped tube, or yellow-topped serum separator tube. Spin samples to separate serum. Save the serum in at least two aliquots. Label tubes as acute serum, with other information listed in Specimen Labeling (e.g., case ID number, date of collection). Store half of the serum at the state health department and send half to CDC for testing.
2. Obtain a follow-up serum sample 4 to 6 weeks after collection of initial serum sample. Collect 7 to 10 cc of patient blood into a marble-topped tube, or yellow-topped serum separator tube. Spin samples to separate serum. Save the serum, and label the tube as convalescent serum with other information listed in Specimen Labeling (e.g., case ID number, date of collection). Send this second serum specimen, together with the remainder of the initial sample stored at the health department, to CDC for paired sera testing.

Note: For pediatric patients, a minimum of 1 cc of whole blood is needed for testing. If possible, collect 1 cc in both an EDTA and clotting tube. However, if only 1 cc can be obtained, use a clotting tube for collection.

VI. Stepwise Collection Procedures for Contacts of Persons with Monkeypox

Blood samples

1. Obtain an initial serum sample when the person is first identified. Collect 7 to 10 cc of patient blood into a marble-topped tube, or yellow-topped serum separator tube. Spin samples to separate serum. Save the serum in at least two aliquots. Label tubes as acute serum, with other information listed in Specimen Labeling (e.g., case ID number, date of collection). Store half of the serum at the state health department and send half to CDC for testing.
2. Obtain whole blood sample. Collect 3 to 5 cc of blood into a purple-topped tube. Gently mix blood with anticoagulant in tube to prevent clotting. Label tube as whole blood, with other information listed in Specimen Labeling (e.g., case ID number, date of collection). Send the tube with whole blood to CDC for testing.
3. Obtain a follow-up serum sample (4 to 6 weeks after collection of the initial serum). Collect 7 to 10 cc of patient blood into a marble-topped tube, or yellow-topped serum separator tube. Spin samples to separate serum. Save the serum, and label the tube as convalescent serum, with other information listed in Specimen Labeling (e.g., case ID number, date of collection). Send convalescent-phase serum, together with the remainder of the acute-phase sample stored at the health department, to CDC for paired sera testing.

Note: Blood samples from person with severe, dense rash may be difficult to draw as the skin may slough off. A central line may be needed for access in cases where a peripheral blood draw is difficult.
Note: For pediatric patients, a minimum of 1 cc of whole blood is needed for testing. If possible, collect at least 1 cc in each of an EDTA and clotting tube. However, if only 1 cc can be obtained, use a clotting tube for collection.

VII. Stepwise Collection Procedure for Patients Suspected to be in the *Prodromal* Phase

1. Tonsillar tissue: Swab or brush posterior tonsillar tissue, then break off the applicator tip into a 1.5- to 2-mL screw-capped tube. ***Do not add transport medium. Use polyester or Dacron swab.***
2. Nasopharyngeal swab: Swab nasopharynx to obtain nasopharyngeal secretions.
3. Blood samples.
    a. Obtain an acute-phase serum sample. Collect 7 to 10 cc of patient blood into a marble-topped tube, or yellow-topped serum separator tube. Spin samples to separate serum. Save the serum in at least two aliquots. Label tubes as acute serum, with other information listed

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in Specimen Labeling (e.g., case ID number, date of collection). Store half of the serum at the state health department and send half to CDC for testing.

b. Obtain whole blood sample. Collect 3 to 5 cc of blood into a purple-topped tube. Gently mix blood with anticoagulant in tube to prevent clotting. Label tube as whole blood, with other information listed in Specimen Labeling (e.g., case ID number, date of collection). Send the tube with whole blood to CDC for testing.
c. Obtain a convalescent-phase serum sample (4 to 6 weeks after collection of acute-phase serum). Collect 7 to 10 cc of patient blood into a marble-topped tube, or yellow-topped serum separator tube. Spin samples to separate serum. Save the serum, and label the tube as convalescent serum, with other information listed in Specimen Labeling (e.g., case ID number, date of collection). Send convalescent-phase serum, together with the remainder of the acute-phase sample stored at the health department, to CDC for paired sera testing.

VIII. Post-collection Procedures

1. After specimen collection is completed, protective equipment worn by the specimen collector should be removed. Disposable equipment (e.g., gown, gloves, respirator, or mask) should be placed in a biohazard bag for disposal with other medical waste. Reusable equipment (e.g., goggles, faceshield) should be decontaminated and set aside for reprocessing. If cloth gowns are used, they should be placed in a bag with other contaminated linen in the patient's room.
2. Needles and other sharp instruments should be placed in a sharps container.
3. Contaminated waste generated through patient care should be handled in accordance with existing facility procedures and local or state regulations for regulated medical waste.
4. Place specimens from a single patient into a biohazard bag with an outside label that includes:
    a. Patient name
    b. Date of collection
    c. Patient date of birth.
5. Package specimens from a single patient (except biopsy specimens):
    a. On gel packs at 4°C;
    b. Place blood tubes (plastic or glass) in individual styrofoam holders;
    c. Place all specimens in appropriate biosafety shipping containers in a manner to withstand all shocks, pressure changes, or other conditions incident to ordinary handling in transportation; and in a manner to avoid leakage of contents.
6. Package non-formalin-fixed lesion biopsy specimens for shipping on dry ice, leave formalin-fixed biopsy specimens at room temperature. Do not freeze formalin-fixed biopsy sample.

Specimens may be stored in conditions outlined above if shipped within 24 hours of collection. If this is not possible, store all samples except EM grids, formalin-fixed tissue, and serum on dry ice or at −2°C to −70°C until, and through, shipment. EM grids, formalin-fixed tissue, and serum should be kept at 4°C until, and through shipment. If there will be a delay in shipping, spin serum in marble or yellow-top tubes to separate from clot, store at 4°C, and ship at 4°C.

IX. Shipping Diagnostic Specimens to CDC

1. For information about shipping specimens to CDC, see Instructions for Packaging and Transport of Diagnostic Specimens for Monkeypox Laboratory Testing at the following URL: www.cdc.gov/ncidod/monkeypox/pdf/specimenguide.pdf

2. Label the package as follows:
    Centers for Disease Control and Prevention
    1600 Clifton Road, NE
    ATTN: STAT Lab (forward to Poxvirus Section)
    Atlanta, GA 30333

3. Shipping and specimen information should be submitted through CDC's Web-based incoming specimen tracking system by completing the form at www.cdc.gov/ncidod/monkeypox/specimentrackingform.htm.

4. Prior to shipping the specimen, please send an e-mail to eoclogistic@cdc.gov to alert CDC that the shipment is in transit.